IFN Alfa-2B and BCG Therapy is an Effective Method in Superficial Bladder Carcinoma

İFN Alfa-2B ve BCG Kombinasyon Tedavisi Yüzeyel Mesane Kanserinde Etkili Bir Metotdur

Yüzeyel Mesane Tümörlerinde İntravezikal Tedavi
Intravesical Treatment for Superficial Bladder Tumors

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Özet

Amaç: Yüzeyel mesane tümörlerinin başlangıç tedavisi tümörün transüretral rezeksiyonudur. Başarılı rezeksiyonlara rağmen, %60-79 rekürrens ve %15 progrsyon oranları vardır. Yüzeyel mesane kanserlerinde ek tedailer önerilmiştir. Biz çalışmamızda yüzeyel mesane kanserli hastalarda tek başına interferon alfa-2b tedavisini interferon alfa-2b ve Bacillus Calmette Guerin (BCG) kombinasyon tedavisi ile birlikte idrar interlökin (IL) 2,6 ve 10 seviyelerini değerlendirmeyi amaçladık. Gereç ve Yöntem: Prospektif çalışmamıza hastanemizde 2004-2007 yılları arasında TUR-MT operasyonu uygulanan hastalar (patolojik evre Ta-T1) dahil edildi. Hastalara TUR-MT operasyonundan 15 gün sonra başlamak üzere, 6 hafta süreyle haftada bir kez ve sonrası 6 ay boyunca aylık intravezikal immünoterapi uygulandı. IL seviyeleri ölçüldü. Bulgular: İntravezikal tedavinin 2. ve 4. saatlerinde idrar IL 2, IL 6 ve IL 10 seviyeleri ölçüldü. 2. ve 4. saatlerde tek başına IFNα-2b ve IFNα-2b+BCG kombinasyon tedavisi alan gruplarda IL 2 seviyelerinde istatistiksel olarak anlamlı farklılık saptandı. (p=0.05) IFNα-2b+BCG kombinasyon grubunda evreler göz önüne alınınca IL 2 ve IL 6 seviyelerinde istatistiksel olarak anlamlı farklılık saptandı. (p=0.05) G3 tümörü olan hastalarda 2. ve 4. saatte IL 2 seviyeleri daha yüksekti (p=0.05), fakat intravezikal tedavi şeklinden bağımsız olarak bu grup hastalarda IL 6 ve IL 10 seviyelerinde anlamlı farklılık yoktu. (p=0.05) Sonuç: Yüzeyel mesane tümörlerinin tedavisinde IFNα-2b ve BCG kombinasyon tedavisi güvenilir ve etkili bir tedavi yöntemidir.

Anahtar Kelimeler

BCG; İnterferon; İnterlökin; İntravezikal Tedavi; Mesane Kanseri

Abstract

Aim: The initial therapy for superficial bladder carcinoma is the transurethral resection of the tumor. In spite of successful resections, there are 60-79% recurrence and 15% progression rates. Additional therapies are suggested for the treatment of superficial bladder carcinoma. We compared the efficacy of interferon alfa-2b monotherapy with interferon alfa-2b plus Bacillus Calmette Guerin (BCG) combination therapy with urine interleukin (IL) 2, 6 and 10 levels of patients with superficial bladder carcinoma. Material and Method: The patients who underwent TUR-BT for superficial bladder tumor (pathological staging Ta-T1) between 2004 and 2007 at our hospital included in this prospective study. Intravesical immunotherapy was administered once a week for 6 weeks and there after a month for 6 months, starting 4 weeks after TUR-BT. IL levels were measured. Results: IL-2, IL-6 and IL-10 levels in urine samples were taken at 2nd and 4th hours of intravesical therapy. A statistically significant difference was observed between mean urine IL-2 levels of patients treated with IFNα-2b monotherapy and IFNα-2b plus BCG combination both at 2nd and 4th hours. (p=0.05) In IFNα-2b plus BCG combination group, there was a statistical significant difference between stages regarding IL-2 and IL-6 levels (p=0.05). Among patients with G3 tumors, IL-2 levels were higher at 2 and 4 hours (p=0.05) but there was no significant difference in IL-6 and IL-10 levels in this group of patients regardless of intravesical therapy received (p=0.05). Discussion: IFNα-2b and BCG combination therapy is a reliable and effective therapy in the management of superficial bladder tumors.

Keywords

BCG; Bladder Carcinoma; Interferon; Interleukin; Intravesical Therapy

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Introduction

Bladder carcinoma is the second most common malignancy in the urogenital system which can occur as a heterogeneous disease varying from superficial low grade tumors to invasive or metastatic high grade tumors [1-3]. The initial therapy for superficial bladder carcinoma is the transurethral resection of the tumor (TUR-BT). This operation is not only curative, especially in low stage and grade tumors (Ta, G1, G2) but also necessary for staging in order to administer additional therapies. However, in spite of successful resections, there are 60-79% recurrence and 15% progression rates and therefore, additional therapies are suggested for the treatment of superficial bladder carcinoma [1,2].

Material and Method

The patients who underwent TUR-BT for superficial bladder tumor (pathological staging Ta-T1) between 2004 and 2007 at our hospital included in this prospective study.

We compared the efficacy of interferon alfa-2b monotherapy with interferon alfa-2b plus Bacillus Calmette Guerin (BCG) combination therapy with urine interleukin (IL) 2, 6 and 10 levels of patients with bladder carcinoma. The patients either received 80 million units of human recombinant IFNα-2b (Schering-Plough Corporation, Kenilworth, NJ, USA) or 60 million units of IFNα-2b with 1/3 standard dose of Connaught Strain BCG (27mg) diluted in 50cc of saline (Connaught Laboratories Limited, Willowdale, Ontario, Canada).

Patient evaluation:

After taking a careful history, a physical examination was given in addition to serum multiple analysis and complete blood count. The localization, size and quantity of the tumors were evaluated with the cystoscopy, performed under general anesthesia prior to TUR-BT assessed after pathological evaluation. Those patients with previous TUR-BT, recurrent bladder tumor and a history of previous intravesical therapy were excluded.

Intravesical Immunotherapy:

Immunotherapy was administered once a week for 6 weeks starting 4 weeks after TUR-BT and there after every month for 6 months. Urine samples were obtained prior to procedure and the intravesical therapies were given after insertion of a 16F catheter. The patients either received 80 million units of human recombinant IFNα-2b (Schering-Plough Corporation, Kenilworth, NJ, USA) or 60 million units of IFNα-2b with 1/3 standard dose of Connaught Strain BCG (27mg) diluted in 50cc of saline (Connaught Laboratories Limited, Willowdale, Ontario, Canada).

The patients were instructed not to urinate for 2 hours and to change their positions while lying down in order to provide full contact of the complete bladder mucosa with the therapeutic solution administered. Urine samples, obtained 2 and 4 hours after each administration, were kept at -20oC until the assay.

Histopathological Examination:

The TUR-BT specimens were stained with hematoxylen-eosin, examined histologically and staged using TNM classification modified in 1997 by AJCC/UICC (1). Mostofi grading system was used in order to determine the pathological grade (Grade 1-3) [1].

Measurement of Interleukin levels:

Samples were evaluated with commercial ELISA kits (Biosource International-human IL-2, human IL-6 human IL-10). For IL measurement, microtubes with their wall rolled up by spesific monoclonal antibodies were used.50 µL of the sample is dealed with the fix solution at the room temperature and inseminated. IL antibodies are tied with immobile antibodies and washed for four times after aspiration. Then these are inseminated with 100 µL biotin conjugate and washed for four times after aspiration again. After that 100 µL Streptavidin-HRP is added and must wait for 30 minutes at the room temperature. By this time the samples are tied to biotin covered antibodies to develop the four layer sandwich model. After aspiration and four times wash up, substrate solution is added for enzymatic reaction to show a colour and fixed 100 µL stop solution to evaluate at 450 nm.

Statistical Analysis:

Statistical analysis was performed with Wilcoxon test and Mann-Whitney U tests using a SPSS 10.0 (Statistical Package For Social Sciences, Chicago, Illinois, USA). Non-parametric tests were used as the number of the patients were less than 30 in groups. For the patients reached only IFNα-2b and IFNα-2b+BCG, two sample tests coupled with Wilcoxon were used to evaluate IL-2, IL-6, IL-10 at second and fourth hour. Mann-Whitney U test was used to evaluate the values IL-2, IL-6, IL-10 IFNα-2b, BCG and IFNα-2b+BCG treatment. $p=0.05$ and $p<0.05$ was accepted as statistically significant.

Results

A total of 40 patients with transitional epithelial bladder carcinoma were included. There were 10 female (25%) and 30 male (75%) patients, with a mean age of 64.6±6.1 (range 51-74). Of these patients, 14 (35%) received 80 million units of IFNα-2b while remaining 26 patients (65%) received 27mg of BCG in addition to 60 million units of IFNα-2b as intravesical therapy as described above at previously indicated doses and duration. Tumor characteristics of those patients are presented in Table 1.

Mean IL-2, IL-6 and IL-10 levels in urine samples taken at 2nd and 4th hours of intravesical therapy were shown in Table 2. A statistically significant difference was observed between mean urine IL-2 levels of patients treated with IFNα-2b monotherapy and IFNα-2b plus BCG combination both at 2nd and 4th hours ($p=0.05$). There was no difference between IL-6 or IL-10 levels at any time point ($p=0.05$).

There was no difference in IL-2, IL-6 and IL-10 levels in patients who received IFNα-2b regardless of the stage of the diseases ($p=0.05$). In IFNα-2b plus BCG combination group, there was a statistical significant difference between stages regarding IL-2 and IL-6 levels but no difference in IL-10 levels in urine samples, taken at 2 and 4 hours after the administration. Among patients with G3 tumors, IL-2 levels were higher at 2 and 4 hours ($p=0.05$) but there was no significant difference in IL-6 and IL-10 levels in this group of patients regardless of intravesical therapy received ($p=0.05$) (Table 2). However, there was a statistical significant difference between 2nd and 4th hour of IL-2, IL-6 and IL-10 levels in both IFNα-2b monotherapy and IFNα-2b plus BCG combination therapy group ($p=0.05$).

Discussion

Despite of a successful resection, 60-70% of superficial bladder tumors may reoccur and 15% of these progresses. After each recurrence, there is a possibility to end up with a higher grade and stage tumor [1]. Grade, recurrence rate and tumor size (≥4 cm) are important for muscle invasion according to the

multivariant analysis [1-2]. There are three risk factors for recurrence and progression after the transurethral resection of the tumor to decide whether further treatment is needed. Low risk group comprise solid, primary or recurrent TaG1-G2 tumors; intermediate risk group comprise multiple, primary or recurrent TaG1-G2 or T1G2 tumors; and high risk group comprise solid or multiple, primary or recurrent or TaG3 tumors with carcinoma insitu (CIS) and T1G3 tumors [4].

BCG seems to be the most effective agent for the prophylaxis of superficial bladder tumors despite of its side effects [5,6]. Most commonly seen side effect of it is cystitis (90%) together with hematuria. Others are fever (3%), hepatitis and pneumonia (0.7%), granulomatous prostatitis (1.3%), arthritis and artralgia (0.5%) and bladder contracture (0.2%). BCG septicemia which is the most serious complication is seen 0.4% [7].

Interferons (IFN) are glycoproteins elaborated in response to the antigenic stimulation. They have anti-tumor effects through its nucleotide synthesis inhibiting, cytokine stimulating and anti-angiogenesis effects [8,9]. Although there is no consensus for the optimal dose, 50-100 million units are administered [10]. Flu-like symptoms may occur in 17% of cases. IFN can eradicate tumor in 20-43% of patients and have a success rate of 20-60% on BCG resistant tumors.

After the introduction of BCG-IFNα-2b combination therapy, it has been recorded that it is much more effective than BCG monotherapy, with a similar anti-tumor efficacy at lower BCG doses. Stricker et al [11] used 60 mg of Pasteur strain (1/2dose) in combination therapy showing that it is well tolerated and successful in patients with papillary tumors and CIS. Another randomized trial showed that combination therapy of IFNα-2b (10 MIU) +1/2 dose BCG (Pasteur) has lower recurrence rates compared with the standard dose of BCG therapy (22% vs 8%) and the adverse effects related with BCG are lower [12]. O'Donnell et al recorded that after a follow-up of 30 months disease free rates after BCG treatment were 63% at 12 months and 53% at 24 months, respectively.

The effect of BCG on stimulating the immune response is not completely understood yet. Animals without T-cells have no response to BCG [13]. Patients under BCG therapy excrete some IL-2 in their urine samples. Several clinical studies showed that urinary IL-2 levels correlate with recurrence with in superficial bladder tumors [14]. Combination of IFNα-2b and BCG results in a stronger immune response [15]. BCG stimulates the infiltration of lymphocytes and Natural Killer cells into the bladder wall [16]. Activated T-helper type 1 cells produce IL-2 and IFN-γ, which results as clinical response [17]. On the other hand, IFNα-2b stimulates cellular immune response, in addition to increasing human leukocyte antigen (HLA) expression in bladder tumor cells. The result is increased lysis of tumor cells by activated cytotoxic T lymphocytes [16].

During intravesical therapy, earliest increase in urinary IL-2 levels occurs after 4th administration. It generally reaches its peak levels at 4-6 hours of the 6th administration and disappears 24 hours later [18]. In this study, intravesical IFNα-2b was used for low risk patients (stage TaG1, TaG2, T1G1) and intravesical combination of IFNα-2b and BCG were used for higher stages and grades. We observed a statistically significant difference among the IL-2 levels in each group. In combination therapy group, urinary IL-2 levels were higher both at 2 and 4 hours after the administration.

After BCG therapy, not only IL-2 but also IL-1, IL-4, IL-6, IL-10 and TNF levels increase [8,19]. IL-6 is an inflammatory cytokine and promotes IL-2 production, T cell proliferation and cytotoxic T cell differentiation [20]. Increased IL-6/IL-10 rate is a positive indicator of antitumor response [21]. We did not find any statistically significant difference in IL-6 levels between groups, but there was a statistically significant difference in IL-6 levels between the 2nd and the 4th administrations.

IL-10 is an agent which provides immunosupression by decreasing the cytokine levels, suppressing the antigen presentation and blocking IL-2 proliferation [22,23]. In-vitro assays show that, tumor resection and survival increases in parallel to a decrease in IL-10 levels [24]. There was no difference in IL-10 levels between groups regardless of tumor grade and tumor stage in our study.

Tumors with higher malignant potential produce higher levels of IFNα (10). In our study we observed that IL-2 and IL-6 levels were higher as the grade increased in both therapy groups regardless of stage.

The limitations of this study can be listed as the small number of cases and the lack of a randomization procedure done in selection of the patients in treatment groups. However, we believe that, in spite of these limitations the results of our study will be helpful to the further studies performed about this issue.

Conclusion

IFNα-2b and BCG combination therapy is a reliable and effective therapy, in the management of superficial bladder tumors, especially reflected by an increase in urinary IL-2 levels which is the most important mediator in response to immunotherapy. Further studies with larger number of patients are needed to elucidate this issue.

References

1. Catalona W, Messing EM. Campbell's Urology. In: Walsh PC, Retik AB, Vaughan ED, Wein AJ, editors. Urothelial tumors of the urinary tract. 8th ed. Philadelphia: Saunders; 2002. vol-4 p. 2732.
2. Turkeri L, Baltacı S, Beduk Y. Temel Üroloji. Mesane tümörleri. 1.Baskı. Ankara: 1998. p.707-26.
3. Peter RC. Smith's General Urology. Appleton and Lange, editors. Urothelial carcinoma cancers of the bladder,ureter and renal pelvis. 14th ed. San Francisco: 1995. p. 353.
4. The proceedings of the V. congress on progress and controversies in oncological urology, held in Rotterdam, is intravesical BCG superior chemotherapy? The Netherlands, October 1998. p. 223-27.
5. Herr HW, Laudone VP, Whitmore WF Jr. An overwiev of intravesical therapy for superficial bladder tumors. J Urol 1987;138(6):1363-68.
6. Herr HW, Pinsky CM, Whitmore WF Jr, Sogani PG, Oettgen HF, Melamed MR. Experience with intravesical BCG therapy of superficial bladder tumors. Urology 1985;25(2):119-23.
7. Lamm DL, Grifflith JG. Intravesical therapy; Does it effect the natural history of superficial bladder tumors? Semin Urol 1992;10(1):39-44.
8. Zhang Y, Khoo HE, Esuvaranathan K. Effects of bacillus calmette-Guerin and interferon-α2b on cytokine production in human bladder cancer cell lines. J Urol 1999;161(3):977-83.
9. Borden EC, Groveman DS, Nasu T, Reznihoff C, Bryan GT. Antiproliferative activities of interferons against human bladder carcinoma cell lines in vitro. J Urol 1984;132(4):800-3.
10. Giannakopoulos S, Gekas A, Alivizatos G, Sofras F, Becopoulos T, Dimopoulos C. Efficacy of escalating doses of intravesical interferon alpha-2b in reducing recurrence rate and progression in superficial transitional cell carcinoma. Br J Urol 1998;82(6):829-34.
11. Stricker P, Pryor K, Nicholson T,Goldstein D,Golovsky D,Ferguson R, et al. Bacillus Calmette-Guerin plus intravesical interferon alpha-2b in patients with superficial bladder cancer. Urology 1996;48(6):957-62.
12. Bercovich E, Deriu M, Manferrari F ,Irianni G. BCG vs BCG plus recombinant interferon alpha-2b in superficial tumors of the bladder. Arch Ital Urol Androl. 1995;67:257-60.
13. Ratliff TL,Gillen D,Catalona WJ. Requirement of a thymus-dependent immune response for BCG mediated antitumor activity. J Urol 1987;137(1):155-8.
14. Saint F, Patard JJ, Maille P, Soyeux P, Hoznek A, Salomon L, et al. T helper ½ lymphocyt urinary cytokine profiles in responding and nonresponding patients after 1 and 2 courses of bacillus calmette-Guerin for superficial bladder cancer. J Urol 2001;166:2142-47.
15. Luo Y,Chen X, Downs TM, DeWolf WC, O'Donnel MA. IFN-alpha 2B enhances Th1 cytokine responses in bladder cancer patients receiving Mycobacterium bovis

bacillus Calmette-Guerin immunotherapy. J Immunol 1999;162(4):2399-405.
16. Lattime EC, Gomello LG, McCue PA. Murine bladder carcinoma cell present antigen to BCG spesific CD4+ T cells . Cancer Res 1992;52:4286-90.
17. Saint F, Patard JJ, Maille P, Soyeux P, Hoznek A, Salomon L, et al. Prognostic value of a T helper 1 urinary cytokine response after intravesical bacillus Calmette-Guerin treatment for superficial bladder cancer. J Urol 2002;167(1):364-7.
18. De Boer EC, De Jong WH, Steerenberg PA, Aarden LA, Tetteroo E, DeGroot ER, et al. Induction of urinary interleukin-1(IL-1),IL-2, IL-6 and tumor necrosis factor during intravesical immunotherapy with bacillus calmette-Guerin in superficial bladder cancer. Cancer Immunol Immunother 1992;34(5):306-12.
19. Patard JJ, Saint F, Velotti F, Abbou CC, Chopin DK. Immune response following intravesical bacillus calmette-Guerin instillations in superficial bladder cancer:a review. Urol Res 1998;26(3):155-9.
20. Thomson AW. The cytokine handbook , Academic press ltd. London, chapt 8,1994, p.15,18,19.
21. Alexandroff AB, Black J, Esuvaranathan K, James K. Antiproliferatif effect of IL-6 on transitional cell carcinoma of the bladder. Insight into mechanism of bacillus calmette-Guerin immunotherapy. Biochem Soc Trans 1997;25(2):270.
22. Riemensberger J, Böhle A, Brandau S. IFN-gamma and IL-12 but not IL-10 are required for local tumor surveillance in a syngeneic model of orthotopic bladder cancer. Clin Exp Immunol 2002;127(1):20-6.
23. Nadler R,Luo Y, Zhao W, Ritchey JK, Austin JC, Cohen MB, et al. Interleukin –10 induced augmentation of delayed type hypersensitivity enhances Mycobacterium bovis bacillus Calmette-Guerin mediated antitumor activity. Clin Exp Immunol 2003;131(2):206-16.
24. Halak BK, Maguire HC Jr, Lattime EC. Tumor induced interleukin-10 inhibits type 1 immune responses directed at a tumor antigen as awell as a non tumor antigen present at the tumor site. Cancer Res 1999;59(4):911-7.